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Association between ASMT and autistic-like traits in children from a Swedish nationwide cohort

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Abstract:	Persons with autism spectrum disorders (ASDs) often display low levels of melatonin, and it has been suggested that this decrease may be due to low activity of the acetylserotonin O-methyltransferase (ASMT), the last enzyme in the melatonin synthesis pathway. Moreover, genetic variants in ASMT have been associated with autism, as well as with low ASMT activity and melatonin levels, suggesting that the low ASMT activity observed in autism may partly be due to variation within the ASMT gene. In this study, we present a symptom-based approach to investigate possible associations between ASMT and autistic-like traits (ALTs) in the general population. To this end, continuous measures of ALTs were assessed in a nationally representative twin cohort (n=1771) from Sweden and six Single Nucleotide Polymorphisms (SNP) and a duplication of exon 2 to 8 in ASMT were genotyped. Our results show a nominally significant association, in girls, between one SNP (rs5949028) in the last intron of ASMT and social interaction impairments. No significant association, however, was observed with traits related to language impairment or restricted and repetitive behavior. In conclusion, our results support the possible involvement of the ASMT gene in ASDs and our finding that only one of three traits shows association suggests that genetic research may benefit from taking a symptom-specific approach to identify genes involved in autism psychopathology.

ASSOCIATION BETWEEN *ASMT* AND AUTISTIC-LIKE TRAITS IN CHILDREN FROM A SWEDISH NATIONWIDE COHORT

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Abstract

Persons with autism spectrum disorders (ASDs) often display low levels of melatonin, and it has been suggested that this decrease may be due to low activity of the acetylserotonin O-methyltransferase (ASMT), the last enzyme in the melatonin synthesis pathway. Moreover, genetic variants in *ASMT* have been associated with autism, as well as with low ASMT activity and melatonin levels, suggesting that the low ASMT activity observed in autism may partly be due to variation within the *ASMT* gene. In this study, we present a symptom-based approach to investigate possible associations between *ASMT* and autistic-like traits (ALTs) in the general population. To this end, continuous measures of ALTs were assessed in a nationally representative twin cohort (n=1771) from Sweden and six Single Nucleotide Polymorphisms (SNP) and a duplication of exon 2 to 8 in *ASMT* were genotyped. Our results show a nominally significant association, in girls, between one SNP (rs5949028) in the last intron of *ASMT* and social interaction impairments. No significant association, however, was observed with traits related to language impairment or restricted and repetitive behavior. In conclusion, our results support the possible involvement of the *ASMT* gene in ASDs and our finding that only one of three traits shows association suggests that genetic research may benefit from taking a symptom-specific approach to identify genes involved in autism psychopathology.

Keywords: Autism spectrum disorders, Autistic-like traits, Melatonin, ASMT, Polymorphism

Introduction

The most prominent characteristics of autism spectrum disorders (ASDs) are impairments in social interaction and communication, language impairments, and repetitive behaviors [1]. The notion that ASD represents a spectrum of impairments is, to a large extent, recognized among both clinicians and researchers (Wing, 1988). Studies of autistic-like traits (ALTs) have suggested that an ASD diagnosis represents the extreme lower end of normally distributed abilities for social communication (Constantino *et al.*, 2004, Posserud *et al.*, 2006). Moreover, ALTs and ASDs have been shown to share common genetic influences (Lundstrom *et al.*, 2012, Robinson *et al.*, 2011). A theoretical partition into three dimensions of autism, i.e. restricted and repetitive behavior, impairments in social communication and language impairments, has been confirmed in several studies (*for review see* (Happe and Ronald, 2008)), and these dimensions have been shown to be influenced by separate genetic factors when investigated in the general population (Ronald *et al.*, 2011). Moreover it has been demonstrated that girls and boys display different ALTs, both among children with ASD (Mandy *et al.*, 2012) and among those children that do not meet diagnostic criteria for ASD (Dworzynski *et al.*, 2012).

Melatonin is involved in circadian rhythm regulation, including the sleep/wake cycle, but it also has an array of other functions, such as regulation of immune responses and neurodevelopmental processes (Stehle *et al.*, 2011). It is released mainly by the pineal gland during the night and is produced by the conversion of serotonin to N-acetylserotonin by the enzyme arylalkylamine N-acetyltransferase (AA-NAT) followed by the conversion of N-acetylserotonin to melatonin by acetylserotonin methyltransferase (ASMT). AA-NAT is generally considered to be the rate-limiting enzyme but recent studies have suggested that variable expression of ASMT has an important effect on the regulation of melatonin synthesis in humans (Maronde *et al.*, 2011), and that the rate-limiting role partly is taken over by

ASMT during night (Liu and Borjigin, 2005). Melatonin is often used to treat sleep impairments in persons with ASD (Rossignol and Frye, 2011, Malow *et al.*, 2011) and low melatonin levels in ASD have been reported by numerous studies (Ritvo *et al.*, 1993, Nir *et al.*, 1995, Miyamoto *et al.*, 1999, Yamashita *et al.*, 1999, Kulman *et al.*, 2000, Tordjman *et al.*, 2005, Melke *et al.*, 2008, Mulder *et al.*, 2010, Tordjman *et al.*, 2012). In connection to the major hypotheses put forth for autism etiology, *i.e.*, neural growth (Torres-Farfan *et al.*, 2009) and synapse formation (Ishida *et al.*, 2005), melatonin has been demonstrated to modulate neurite outgrowth in cultured neuronal cells (Lavebratt *et al.*, 2010). Taken together, previous findings hence suggest that an impaired melatonin synthesis and/or secretion may be associated with ASDs and related phenotypes. Indeed, it has been demonstrated that the melatonin deficit in persons with autism correlates with low activity of the ASMT enzyme, and, in some patients, are associated with mutations in the *ASMT* gene (Melke *et al.*, 2008). In addition, rare functional mutations in *ASMT* have been identified in persons with ASD (Wang *et al.*, 2013, Jonsson *et al.*, 2010, Toma *et al.*, 2007). Moreover, it has been demonstrated that polymorphisms in the promoter region of *ASMT* influences mRNA transcription and are associated with ASDs (Melke *et al.*, 2008). However, negative results from association studies of *ASMT* polymorphisms have also been published (Toma *et al.*, 2007, Holt *et al.*, 2010, Wang *et al.*, 2013) and none of the Single Nucleotide Polymorphisms (SNP) in *ASMT* reached genome-wide statistical significance for association in the study by Anney et al. (Anney *et al.*, 2010); the only large, genome wide, association study (GWA) on ASD that has included the *ASMT* gene. In addition to the results from mutation screening and association studies, a microduplication (~18 kb) has been identified in the *ASMT* gene and found to be significantly more common in ASDs (5.8%) than in controls (1.6%) (Cai *et al.*, 2008).

Based on previous suggestions of *ASMT* as a candidate gene for autism susceptibility, we have investigated the possible association between polymorphisms in the *ASMT* gene and autistic-like traits in children from the general population.

Materials and methods

Subjects

The Child and Adolescent Twin Study in Sweden (CATSS) is a nationwide cohort that focuses on all Swedish twins turning 9 or 12 years since 2004 (Anckarsater *et al.*, 2011). The CATSS study has an 80% response rate, making it a highly representative population sample (Anckarsater *et al.*, 2011). Data is currently available on 12,446 children: n=5944 for 9-year-olds and 6496 for 12-year-olds. The present study used genetic material from the first DNA collection from CATSS (both 9- and 12-year-olds) including information from 1771 subjects in total (887 girls and 884 boys). Notably, since the sample is recruited from the general population, it includes the full variation of ALTs, *i.e.*, also subjects meeting the criteria for clinical diagnoses of ASD and other neuropsychiatric disorders. Moreover, the focus of our study was to investigate the possible influence of common genetic variation in *ASMT* on ALTs. Hence, 24 subjects were excluded from the analyses due to documented brain damage (most commonly cerebral palsy) or a known genetic syndrome (most commonly Down's syndrome but also fragile X syndrome) since individuals with these conditions are well known to display a high degree of autism-related symptoms (Zafeiriou *et al.*, 2007). The total number of subjects included in the statistical analyses is hence 1747 and consist of 357 monozygotic twin (MZ) pairs, 500 dizygotic (DZ) twin pairs and 33 subjects without their co-twin. To determine twin zygosity a panel of 47 SNPs were used (Hannelius *et al.*, 2007). Notably, although all statistical analyses (see below) were adjusted for kinship, the population is analyzed as a representative sample of children from the general population in Sweden. The CATSS study has ethical approval from the Karolinska Institute Ethical Review Board, and informed consent was obtained from the participants.

Measurements

Parents of all twins were contacted when their twins turned 9 or 12 years and asked to

participate in a telephone interview containing, among other instruments, the Autism–Tics, Attention-Deficit/Hyperactivity Disorder (AD/HD), and Other Co-morbidities inventory (A-TAC) (Hansson *et al.*, 2005, Larson *et al.*, 2010). The A-TAC is a sensitive tool for screening the general population for child autism spectrum disorders and associated conditions and can also be used as a dimensional measure (Larson *et al.*, 2010). ALTs were measured by 17 items in the A-TAC, including 12 questions specifically addressing the DSM-IV symptom criteria for autistic disorder. Each of the 17 items has three response categories; ”no” (coded 0), “yes, to some extent” (coded 0.5), and “yes” (coded 1.0). The measure of total ASD scores is the sum of the 17 A-TAC items related to autism/ASD. Out of these items, six correspond to the language impairment, six to the social interaction impairment and five to the restricted and repetitive behavior module. The A-TAC is freely available from the Internet as an appendix to the published article by Larson *et al.* (2010). The A-TAC has previously been used as a dimensional measure of autistic-like traits in genetic association studies (Walum *et al.*, 2012, Molero *et al.*, 2013) and in several studies to investigate the heritability of (Lundstrom *et al.*, 2012), and relation between, different neurodevelopmental and behavioral problems in children from the general population (Anckarsater *et al.*, 2008, Lundstrom *et al.*, 2011, Lichtenstein *et al.*, 2010). In addition, the A-TAC questionnaire have been shown to be a valid instrument to screen for and to identify cases of ASD and overlapping neuropsychiatric/developmental disorders (Larson *et al.*, 2010).

Polymorphism selection and genotyping

DNA was extracted from saliva samples using OraGene® DNA self-collection kit (DNA Genotek, Inc., Ottawa, Ontario, Canada). Six SNPs in the *ASMT* gene (table 1) were genotyped with the Kompetitive Allele Specific PCR (KASP™) genotyping system (LGC, Kbiosciences, Herts, UK). To select SNPs for association analyses, genotyping data for the *ASMT* gene (including 1kb up- and downstream of the coding region) was downloaded from

the International Haplotype Mapping Project web site (http://www.hapmap.org) for the Caucasian population with European ancestry from the Centre d'etude du polymorphisme humain (CEPH) collection. The data was then incorporated into the Haploview program and the Tagger function within Haploview was used to assign Tag SNPs (Gustafsson *et al.*, 2011). Six SNPs in the *ASMT* gene (rs1128551, rs6644777, rs4446909, rs5989681, rs6588809, and rs5949028) were chosen, by pairwise tagging, to capture the common variations within these genes and the surrounding area with a minimum r^2 of 0.80 (for their location and the SNPs which they tag). SNPs rs4446909 and rs5989681 were force-included based on previous findings (Melke *et al.*, 2008) and the missense SNP rs6588809 in exon 7 of the gene was force-included based on its possible function role. SNPs with a minor allele frequency (MAF) >0.2 in the Caucasian sample were chosen to ensure adequate power given our sample size, which was fixed by external limitations prior to the study. Linkage disequilibrium (LD) in our population, measured by D' values, between the six SNPs are presented in table 2. All SNPs were found to be in Hardy Weinberg Equilibrium ($p > 0.01$), which was calculated by using one subject in each MZ twin pair and both subjects from the DZ twin pairs. The genotyping success rate was over 94% (table 1).

Analysis of the copy number variation in the *ASMT* gene was performed using quantitative polymerase chain reaction (q-PCR). One probe in exon six was chosen based on previous findings showing a duplication in this region in the *ASMT* gene (Cai *et al.*, 2008). The q-PCR-probe was designed using GeneAssist™ Copy Number Assay Workflow Builder (Applied Biosystems) and the reference assay used was the TaqMan® Copy Number Reference Assay RNase P. The assay was run in duplicates and three calibrator samples were used. qPCR analysis was performed using 7900HT Sequence detection system Software v2.4 (Applied Biosystems) and CopyCaller® (Applied Biosystems) was used to analyze the copy number variation results. The genotyping of the duplication had a success rate of over 93% (table 1).

Statistical analysis

Statistical association between six SNPs and the duplication in the *ASMT* gene and continuous measures of ALTs, including the A-TAC modules restricted and repetitive behavior, language impairment and social interaction impairment, were estimated using linear mixed effect models in the MIXED procedure (PROC MIXED) of SAS 9.3 (SAS Institute, Inc., Cary, NC). This model allowed us to adjust for the dependent nature of the twin observations i.e., A-TAC scores from all genotyped subjects were included in the analyses. Specifically, given that MZ twins, on average, share 100% of their genome while DZ twins only share 50% of their genome, and that MZ twins are more similar than DZ twins in ALT scores (Lundstrom *et al.*, 2012), we specified two separate variance-covariance matrices for MZ twins and for DZ twins. The sample size also made it possible to analyze girls and boys separately. Significant p-values were corrected for analyses of six SNPs and three A-TAC domains, using Bonferroni correction for multiple testing. Association analysis of the duplication in the *ASMT* gene was only performed with regards to the total ALT, i.e., not with each module of ALTs nor sex-specific analysis, due to the low frequency of the duplication.

The G*Power software was used to asses effect-size calculations and post-hoc power analysis for the association analyses of the six SNPs and the duplication in the *ASMT* gene. These analyses are based on a significant p-value (0.05/18=0.0028) corrected for six SNPs and three A-TAC modules for the total population, i.e., not corrected for kinship, and for the sex specific analyses.

Results

Association analyses of the six *ASMT* SNPs and A-TAC scores revealed a significant association, in girls, between an intronic SNP (rs5949028, MAF=0.4) and social interaction impairment (p=0.0023, η^2= 0.015), where the C-allele carriers were shown to have higher scores (table 3). Although we did not see significant association between rs5949028 and the two other modules, we could see a trend showing that female C-allele carriers also had higher scores on restricted and repetitive behavior (p=0.052). We did not see any significant associations between the other SNPs and A-TAC scores, however, we could see a trend that girls carrying the G-allele of one of the promoter SNPs (rs4446909) had higher scores on language impairment (p=0.074). No significant associations for any of the studied SNPs were observed in boys. In our study, we had a power of 80% to detect small effect-sizes (η^2=0.01) and a power of 100% to detect medium to large effect sizes (η^2>0.06) for analyses in the total population. For the sex-specific analyses we had a power of 36% to detect small effect-sizes and a power of 100% to detect medium to large effect sizes**.** Effect sizes were determined according to Cohen´s conventional criteria (Zafeiriou *et al.*, 2007).

In our study, we also investigated a microduplication of exon 2 to 8 in the *ASMT* gene, which was found in 27 individuals (1.7%) in our population. All these individuals were shown to have one extra copy of the region investigated, except for one monozygotic twin pair who had two extra copies. This duplication was analyzed with respect to total ASD scores, although no significant associations could be shown (p=0.662). For this analysis, we had a power of 98% in our total sample to detect small effect sizes (η^2>0.01).

Discussion

Biochemical studies have provided evidence for the importance of melatonin in autism related phenotypes. In addition, both mutation screenings (Toma *et al.*, 2007, Melke *et al.*, 2008) and association studies (Melke *et al.*, 2008) have implicated the *ASMT* gene in ASD. The results

from the present study tentatively suggest an association between an intronic *ASMT* polymorphism (rs5949028) and ALTs in children from the general population. Our results do not suggest a major involvement of this polymorphism in ASD since the association was only observed in girls and the effect size of the studied SNP on social interaction impairment scores was small (η^2= 0.015). Previously, the rs5949028 has been investigated in one case-control study of ASD (Holt *et al.*, 2010), with negative results and this polymorphism has not been genotyped in any of the large GWAs targeting common variants affecting risk for ASDs (Anney *et al.*, 2010, Wang *et al.*, 2009, Weiss *et al.*, 2009). Notably, of the large GWAs on ASDs, only Anney and coworkers (Anney *et al.*, 2010) uses a genotyping array (Illumina's 1M Beadchip) that includes any SNPs in the *ASMT* gene at all. In their study, none of the five *ASMT* polymorphisms analyzed reached genome-wide significance for association with ASDs (Anney *et al.*, 2010). However, neither the SNPs investigated in our study, nor the SNPs that have been significantly associated with ASD in previous studies (Melke *et al.*, 2008), were genotyped (Anney *et al.*, 2010). To the best of our knowledge, only one genome wide study investigating dimensional measures of autistic-like traits has been published (Ronald *et al.*, 2010), however, the genotyping array (Affymetrix 500K) used in this study does not genotype any SNPs in *ASMT*.

None of the promoter SNPs (rs4446909 and rs5989681) previously associated with ASD were found to influence ALTs in our study. However, in line with previous findings, we could see that carriers of the G-allele of rs4446909 had slightly higher scores for language impairments in girls (p=0.074).

The result that different SNPs in *ASMT* have been associated in different studies may be due to limited gene coverage or variation in LD-patterns between samples. It is, however, also possible that the promoter SNPs indeed are associated with more severe phenotypic expressions of autism, *i.e.*, ASD diagnosis, whereas the SNP (rs5949028) in our study is more

associated with social behavior in the general population.

Recently, further support for an involvement of the *ASMT* gene in ASD was presented in a Multiplex Ligation-dependent Probe Amplification (MLPA) study showing that a duplication in the *ASMT* gene was significantly more common in ASDs, as compared to controls (Cai *et al.*, 2008), suggesting that the expression of the ASMT protein may be altered in persons with ASD. In our study, however, we could not observe any significant association between this duplication and measures of ALTs.

The A-TAC questionnaire and the relatively large sample size permitted us to investigate the different dimensions of ALTs separately, revealing association between the *ASMT* gene and traits related to impairments in social interaction but neither with restricted/repetitive behavior nor symptoms related to language impairments. Our findings are hence in line with the notion that the different ALTs are influenced by separate sets of genes (Ronald *et al.*, 2011).

The large proportion of female subjects in our study also allowed us to analyze boys and girls separately, which is often not possible in case-control studies with low prevalence of girls with ASDs. The significant association for girls in our study is in line with the suggestion that different mechanisms are involved in ASDs for males and females (Lai *et al.*, 2011). In addition, it has also been suggested that the ASD phenotype differ between boys and girls, and attempts have been made to modify current diagnostic manuals for a sex specific diagnostic criteria (Kopp and Gillberg, 2011).

There are limitations of our study. Most importantly, our sample size is moderate and the small effect size of the *ASMT* polymorphism on ALTs observed in this study obviously does not mean that this polymorphism may serve as a predictor for autism psychopathology. Hence, our results should be interpreted with caution and either previous findings, or our

results, may be coincidental findings. In addition, the associated polymorphism has, to our knowledge, not been investigated functionally, and the intronic position does not implicate a functional effect on the ASMT protein. Our finding may thus reflect an indirect association, *i.e.,* the associated polymorphism could be partly in linkage disequilibrium with a more rare functional variant. However, by demonstrating a modest but significant influence of a SNP in the *ASMT* gene on an autism related phenotype, our results support the possible involvement of *ASMT* as a risk factor for autism susceptibility. Our results also show that not all traits of autism are associated with the investigated gene, suggesting that genetic research may benefit from taking a symptom-specific approach to finding genes associated with autism related phenotypes. In addition, the association appears in girls only, further emphasizing the importance of sex-specific analyses in studies of ASDs. Finally, in our study, no association between the duplication of *ASMT* and autism was found. However, the low frequency of this duplication requires association studies in larger samples and to elucidate its role in autism psychopathology, functional studies are highly warranted.

Conflict of interest

There are no conflict of interest.

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Tables

Table 1. Polymorphisms genotyped in *ASMT.*

Polymorphism	Location	MAF	Alleles	Genotyping success rate
rs1128551	5’	0.45	C/T	0.94
rs6644777	5’	0.28	A/G	0.95
rs4446909	5’	0.20	A/G	0.94
rs5989681	5’	0.24	C/G	0.99
rs6588809	Exon 7	0.41	C/T	0.98
rs5949028	intron 9	0.39	T/C	0.94
Duplication	Exon 2-8	0.01	DUP/no DUP	0.93

Table 2. Linkage disequilibrium (LD), measured by D' values, between the six SNPs in *ASMT.*

	rs1128551	**rs6644777**	**rs4446909**	**rs5989681**	**rs5949028**	**rs6588809**
rs1128551	-	0.007	0.009	0.001	0.092	0.017
rs6644777	0.007	-	0.502	0.531	0.202	0.016
rs4446909	0.009	0.502	-	0.979	0.005	0.036
rs5989681	0.001	0.531	0.979	-	0.057	0.004
rs5949028	0.092	0.202	0.005	0.057	-	0.262
rs6588809	0.017	0.016	0.036	0.004	0.262	-

Table 3. Autistic-like traits, as assessed with the A-TAC, by *ASMT* genotypes.

	Restricted & repetitive behavior			Language impairment			Social interaction impairment		
	Mean A-TAC score (SD)			*Mean A-TAC score (SD)*			*Mean A-TAC score (SD)*		
	All	Boys	Girls	All	Boys	Girls	All	Boys	Girls
rs1128551									
C/C (356)	0.26 (0.73)	0.39 (0.93)	0.15 (0.45)	0.32 (0.73)	0.44 (0.89)	0.22 (0.51)	0.35 (0.80)	0.47 (1.02)	0.24 (0.52)
C/T (783)	0.28 (0.64)	0.36 (0.73)	0.19 (0.52)	0.29 (0.62)	0.40 (0.73)	0.18 (0.46)	0.30 (0.64)	0.37 (0.71)	0.22 (0.54)
T/T (505)	0.33 (0.76)	0.43 (0.86)	0.22 (0.62)	0.37 (0.77)	0.48 (0.92)	0.26 (0.55)	0.38 (0.84)	0.47 (1.00)	0.27 (0.60)
P-value	0.460	0.644	0.436	0.427	0.905	0.138	0.329	0.385	0.468
rs6644777									
A/A (124)	0.29 (0.6)	0.40 (0.72)	0.17 (0.39)	0.39 (0.69)	0.54 (0.81)	0.22 (0.47)	0.33 (0.64)	0.43 (0.71)	0.23 (0.53)
A/G (687)	0.30 (0.73)	0.41 (0.82)	0.20 (0.60)	0.32 (0.72)	0.43 (0.86)	0.21 (0.53)	0.35 (0.78)	0.43 (0.91)	0.27 (0.61)
G/G (854)	0.29 (0.71)	0.38 (0.83)	0.20 (0.53)	0.32 (0.69)	0.41 (0.80)	0.22 (0.52)	0.32 (0.74)	0.41 (0.87)	0.23 (0.56)
P-value	0.950	0.970	0.934	0.623	0.552	0.936	0.570	0.898	0.814
rs4446909									
A/A (72)	0.15 (0.37)	0.32 (0.56)	0.05 (0.16)	0.20 (0.51)	0.50 (0.76)	0.04 (0.14)	0.19 (0.39)	0.38 (0.55)	0.10 (0.22)
A/G (517)	0.31 (0.75)	0.41 (0.89)	0.20 (0.56)	0.31 (0.70)	0.42 (0.87)	0.21 (0.44)	0.34 (0.76)	0.40 (0.91)	0.28 (0.59)
G/G (1052)	0.29 (0.69)	0.37 (0.78)	0.20 (0.57)	0.34 (0.71)	0.43 (0.80)	0.24 (0.57)	0.34 (0.74)	0.42 (0.85)	0.24 (0.58)
P-value	0.315	0.723	0.338	0.454	0.853	0.074	0.373	0.995	0.207
rs5989681									
G/G (1004)	0.29 (0.68)	0.37 (0.78)	0.20 (0.55)	0.33 (0.70)	0.41 (0.79)	0.24 (0.58)	0.32 (0.72)	0.41 (0.85)	0.24 (0.56)
G/C (618)	0.31 (0.75)	0.41 (0.87)	0.20 (0.59)	0.33 (0.70)	0.44 (0.86)	0.21 (0.45)	0.36 (0.8)	0.44 (0.93)	0.29 (0.63)
C/C (103)	0.22 (0.48)	0.45 (0.65)	0.06 (0.16)	0.30 (0.62)	0.52 (0.82)	0.14 (0.35)	0.25 (0.61)	0.47 (0.86)	0.10 (0.22)
P-value	0.690	0.732	0.240	0.834	0.735	0.440	0.358	0.751	0.137

rs6588809									
C/C (284)	0.27 (0.67)	0.39 (0.82)	0.14 (0.41)	0.33 (0.67)	0.48 (0.84)	0.16 (0.33)	0.34 (0.75)	0.46 (0.93)	0.21 (0.45)
T/C (848)	0.27 (0.68)	0.33 (0.76)	0.21 (0.59)	0.29 (0.65)	0.37 (0.73)	0.22 (0.57)	0.29 (0.69)	0.35 (0.79)	0.23 (0.58)
T/T (582)	0.34 (0.75)	0.48 (0.88)	0.18 (0.54)	0.37 (0.77)	0.5 (0.93)	0.23 (0.53)	0.39 (0.81)	0.5 (0.94)	0.29 (0.62)
P-value	0.263	0.127	0.375	0.226	0.239	0.276	0.051	0.153	0.295
rs5949028									
C/C (578)	0.33 (0.81)	0.41 (0.91)	0.26 (0.69)	0.37 (0.78)	0.49 (0.93)	0.25 (0.56)	0.36 (0.77)	0.39 (0.86)	0.33 (0.66)
T/C (843)	0.28 (0.64)	0.36 (0.75)	0.18 (0.47)	0.31 (0.67)	0.40 (0.79)	0.21 (0.51)	0.33 (0.75)	0.43 (0.90)	0.22 (0.53)
T/T (219)	0.23 (0.57)	0.40 (0.75)	0.06 (0.19)	0.25 (0.51)	0.37 (0.64)	0.14 (0.30)	0.27 (0.62)	0.43 (0.81)	0.12 (0.26)
P-value	0.269	0.649	0.052	0.097	0.128	0.137	0.383	0.930	**0.0023 (P_c=0.041*)**

Association analysis for all subjects, boys and girls between genotypes and the different modules of autism spectrum disorders, as assessed by the A-TAC

*P_c=p-value corrected for multiple analyses using the Bonferroni method.